Accession No. 408563-04

**DATA EVALUATION RECORD** 4/14/1989

1. **CHEMICAL:** $^{3}$H-Avermectin $B_1$
   Shaughnessey No. 122804

2. **TEST MATERIAL:** $^{3}$H-Avermectin $B_1$; Lot #L-676,863-164L010; $^{3}$H-avermectin $B_1$ was a 7.95:1 mixture of avermectin $B_{1a}$ and avermectin $B_{1b}$, with the tritium label (at the 5-position) present only in the avermectin $B_{1a}$ fraction.

3. **STUDY TYPE:** Estuarine Organism 96-hour Flow-Through Toxicity Test. Species Tested: Mysid Shrimp (Mysidopsis bahia)

4. **CITATION:** Suprenant D.C. (1988) Acute Toxicity of $^{3}$H-Avermectin $B_1$ to Mysid Shrimp (Mysidopsis bahia) Under Flow-Through Conditions. Prepared by Springborn Life Sciences, Wareham, Massachusetts. Submitted by Merck and Company, Inc., Rahway, New Jersey. Accession No. 408563-04.

5. **REVIEWED BY:**

   Kimberly D. Rhodes
   Aquatic Toxicologist
   Hunter/ESE, Inc.

   **Signature:**

   **Date:**

6. **APPROVED BY:**

   Prapimpan Kosalwat, Ph.D.
   Staff Toxicologist
   KBN Engineering and
   Applied Sciences, Inc.

   **Signature:**

   **Date:** Daniel Rieder 4-14-89

   Henry T. Craven, M.S.
   Supervisor, EEB/HED
   USEPA

   **Signature:**

   **Date:** Henry T. Craven

7. **CONCLUSIONS:** This study appears scientifically sound and fulfills the Guideline requirements for a 96-hour flow-through acute toxicity study for estuarine and marine shrimp. The 96-hour LC50 based upon mean measured concentrations of $^{3}$H-Avermectin $B_1$ to the mysid (Mysidopsis bahia) was 22 ng/L. Therefore, $^{3}$H-Avermectin $B_1$ is classified as very highly toxic to the mysid.

8. **RECOMMENDATIONS:** N/A

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: The mysids used in this toxicity test were cultured and acclimated at the testing facility. Prior to testing, mysids were maintained in natural filtered seawater under recirculating conditions. Mysids were fed brine shrimp nauplii two times daily and Hatchfry Encapsulon$^{R}$ three times weekly. The mysid culture area received a regulated photoperiod of 16-hours light and 8-hours darkness. Commercial aquarium heaters were used to maintain the culture solution temperatures at 25 $\pm$ 1$^{o}$C. The maximum test organism biomass was < 3 mg/L at any given time during the test.

B. Test System: The test was conducted using an exposure system consisting of a modified Mount and Brungs (1967) proportional diluter, a temperature controlled water bath, and a set of 14 test aquaria. The test system was designed to provide five concentrations of test material, a dilution water (seawater) control and solvent control. The solvent control solution contained the maximum amount of acetone present in any test concentration (2.2 uL/L). Each glass test aquarium measured 39 x 20 x 25 centimeters (cm) with a self-starting siphon attached to a system drain. Two mysid retention chambers, constructed from glass petri dishes and nylon screen (363-um mesh size opening), were positioned in each aquarium. This system allowed the aquarium volume to fluctuate between 3.1 and 7.0 L. The flow rate of exposure solutions to each test aquaria was equivalent to 7-volume additions per 24 hours. Test aquaria were impartially positioned in a water bath containing circulating water heated by immersion coil heaters and regulated by a mercury column thermoregulator designed to maintain the test solution temperature at 25 $\pm$ 1$^{o}$C.

C. Dosage: 96-hour acute flow-through test.

D. Design: Selection of $^{3}$H-Avermectin $B_1$ concentrations for the 96-hour acute toxicity test with mysid shrimp was based on preliminary exposures of M. bahia to $^{3}$H-Avermectin $B_1$. The test was initiated when 20 ($\leq$ 24-hours old) mysid shrimp were randomly distributed to each concentration or control (10 mysids per replicate). A control, solvent control and nominal $^{3}$H-Avermectin $B_1$ concentrations of 4.5, 6.9, 11, 16, and 25 ng/L were maintained. All concentrations were observed once every

24 hours for mortality and abnormal effects. The water quality parameters (dissolved oxygen, pH, salinity, and temperature) were measured and recorded daily for each replicate of the control solutions and each treatment level. Test solution temperature was also continuously monitored in one replicate of the solvent control solution throughout the study. Analytical determination of $^{3}$H-Avermectin $B_1$ was performed on all treatment levels at 0 and 96 hours using radiometric analysis.

E. Statistics: The mean measured concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence intervals for each 24-hour interval of the exposure period. LC50 values were empirically estimated as being greater than the highest concentration tested when no test concentrations caused 50% or more mortalities. If at least one test concentration caused mortality of greater than or equal to 50 % of the test population, then a computer program (Stephan, 1977, 1982) was used to calculate the LC50 values and 95% confidence intervals.

12. REPORTED RESULTS: Analytical determination of test concentrations resulted in mean measured concentrations of 4.2, 7.7, 10, 16, and 29 ng/L. The mean measured concentrations were 91 to 116% of the nominal concentrations. "The mean measured test concentrations, the corresponding mortalities and the observations made during the 96-hour test are presented in Table 3 (attached). After 96 hours of exposure mortality was observed among 80, 15, and 15% of the mysids at the three highest mean measured test concentrations (29, 16 and 10 ng/L $^{3}$H-Avermectin $B_1$, respectively). Mortality of $\leq$ 5% was observed among mysids exposed to the remaining treatment levels (7.7 and 4.2 ng/L $^{3}$H-Avermectin $B_1$). Based on these data, the 96-hour LC50 (95% confidence interval) for $^{3}$H-Avermectin $B_1$ and mysids was calculated to be 22 (16 - 29) ng/L. Mortality among control organisms during the study was $\leq$ 10%. Based on criteria established by U.S. EPA (1985), $^{3}$H-Avermectin $B_1$ would be classified as very highly toxic to mysid shrimp." The 24-, 48-, 72-, and 96-hour LC50 values for mysids exposed to $^{3}$H-Avermectin $B_1$ was estimated to be >29, >29, 33, and 22 ng/L based on mean measured concentrations. The no observed effect concentration (NOEC) at 96-hours was 7.7 ng/L based on mean measured concentrations.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 24-, 48-, 72-, and 96-hour LC50 values for mysids exposed to $^{3}$H-Avermectin $B_1$ was estimated to be >29, >29, 33, and 22 ng/L based on mean measured concentrations. The no observed effect concentration (NOEC) at 96-hours was 7.7

ng/L based on mean measured concentrations.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

o The SEP states that natural or reconstituted seawater of 10 to 17 °/oo salinity should be used when testing euryhaline shrimp species. The natural seawater used during the toxicity study had a salinity of 30 °/oo.

o The SEP states that most shrimp are to be tested at 22°C and the actual measured temperature should not deviate more than 1°C during the test. During this study the test temperature, measured in one replicate of the solvent control and in the 11 ng/L solutions, ranged from 23 - 25°C.

o The SEP states that a test is not acceptable if more than 5% of the control organisms die during a flow-through system. During the toxicity study, 10 percent of the solvent control organisms died.

The toxicity report did not provide the following information required by the SEP:

o The SEP recommends a 16-hour light and an 8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark. The report did not state whether a 15- to 30-minute transition period between light and dark was maintained.

o The active ingredient of the test substance was not reported. However, the information provided by the EEB indicated that the active ingredient was 99%.

B. Statistical Analysis: The reviewer used the Toxanal computer program to calculate the LC50 values. These calculations are attached. The probit method provides a 96-hour LC50 value of 22 ng/L with a 95 percent confidence interval of 19 to 28 ng/L which is similar to that reported by the author. The slope of the toxicity curve was estimated to be 5.2.

C. Discussion/Results: The study results appear to be scientifically valid, however, solvent control mortality exceeded the 5 percent limit. The 96-hour LC50 value

based upon mean measured concentrations was estimated to be 22 ng/L. Therefore, $^{3}$H-Avermectin $B_1$ is classified as very highly toxic to the mysid, Mysidopsis bahia.

D. Adequacy of the Study:

(1) **Classification:** Core

(2) **Rationale:** Solvent control mortality which exceeded the 5 percent limit, is not a severe enough deficiency to invalidate the study.

(3) **Repairability:** N/A

15. COMPLETION OF ONE-LINER: Yes, 2/8/89.